FIGURE 1

A. CTTATCGATACCGTCGAAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCAT

CACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCA
 +++++++ ↑ +++++++++++

ATGTATCTTATCATGTC   **(Seq ID NO:1)**   <u>Cleavage site</u>

B.

AAUAAA
+++++
━━━━━━━━━━━━━━━━━━━━━GCA

C.

(Seq ID NO:18)

━━━━━━━━━━━━━━━━━━━━━GCAaaaaaaaaaaaaaaaaaaaaaa

+ Upstream and downstream
   cleavage- polyadenylation elements

FIGURE 2

▶ ITR
CATCATCAAT   AATATACCTT   ATTTTGGATT   GAAGCCAATA   TGATAATGAG   GGGGTGGAGT   60
                                      ITR ◀   ▽
TTGTGACGTG   GCGCGGGGCG   TGGGAACGGG   GCGGGTGACG   TAGTAGTGTG   GCGGAAGTGT   120
                                      AP3 DNA BS   • • • • • •
GATGTTGCAA   GTGTGGCGGA   ACACATGTAA   GCGACGGATG   TGGCAAAAGT   GAC G TTTTTG   180
          ▽   ▶Ψ   • • • • • •   •
GTGTGCGCCG   GTGTACACAG   GAAGTGACAA   TTTTCGCGCG   GTTTTAGGCG   GATGTTGTAG   240
          • •   • • • • • • • •   XXXXXXXX   •   • • • • •   +
TAAATTTGGG   CGTAACCGAG   TAAGATTTGG   CCATTTTCGC   GGGAAAACTG   AATAAGAGGA   300
+++ +++++   +   +++++++++   XXXXXXX   X   ▼•• ••
AGTGAAATCT   GAATAATTTT   GTGTTACTCA   TAGCGCGTAA   TATTTGTCTA   GGGCCGCGGG   360
•••+++ ++++ +++++ +++++   +   ++++   ++++++   ++
GACTTTGACC   GTTTACGTGG   AGACTCGCCC   AGGTGTTTTT   CTCAGGTGTT   TTC CGC GTTC   420
+++++++++++ +++++   E1a TATA Box
CGGGTCAAAG   TTGGCGTTTT   ATTATTATAG   TCAGCTGACG   TGTAGTGTAT   TTA TAC CCGG   480
                 +1▶
TGAGTTCCTC   AAGAGGCCAC   TCTTGAGTGC   CAGCGAGTAG   AGTTTTCTCC   TCC GAG CCGC   540
          ▽
TCCGACACCG   GGACTGAAA A   TGAGACATAT   TATCTGCCAC   GGAGGTGTTA   TTACCGAAGA   600

• Enhancer elements   ▽—▽ dl 103-551 **Ar6**
X E2F-motif   ▽—▽ dl 189-551   (SEQ ID NO:2)
+ Packaging elements   ▼—▼ dl 357-551 **Ar5**

FIGURE 3A

```
  1  CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGGGGGTGGAGT
     +-------------------------ITR----------------------------

 61  TTGTGACGTGGCGCGGGGCGTGGGAACGGGGCGGGTGACGTAGGGCGCGATCAAGCTTAT
     +-------------------------ITR-----------+          +----

121  CGATACCGTCGAAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATC
     --------------------------polyA--------------------------

181  ACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTC
     --------------------------polyA--------------------------

241  ATCAATGTATCTTATCATGTCTGGATCCGCGCCGCTAGCGATCATCCGGACAAAGCCTGC
     -------------------+           +----------------

301  GCGCGCCCCGCCCCGCCATTGGCCGTACCGCCCCGCGCCGCCGCCCCATCTCGCCCCTCG
     -----------------------E2F-1 promoter--------------------

361  CCGCCGGGTCCGGCGCGTTAAAGCCAATAGGAACCGCCGCCGTTGTTCCCGTCACGGCCG
     -----------------------E2F-1 promoter--------------------

421  GGGCAGCCAATTGTGGCGGCGCTCGGCGGCTCGTGGCTCTTTCGCGGCAAAAAGGATTTG
     -----------------------E2f-1 promoter--------------------

481  GCGCGTAAAAGTGGCCGGGACTTTGCAGGCAGCGGCGGCCGGGGGCGGAGCGGGATCGAG
     -----------------------E2f-1 promoter--------------------

541  CCCTCGATGATATCAGATCATCGGATCCCGGTCGACTGAAAATGAGACATATTATCTGCC
     --------------+           +------------------------

601  ACGGAGGTGTTATTACCGAAGAAATGGCCGCCAGTCTTTTGGACCAGCTGATCGAAGAGG
     -----------------------E1a gene--------------------------

661  TACTGGCTGATAATCTTCCACCTCCTAGCCATTTTGAACCACCTACCCTTCACGAACTGT
     -----------------------E1a gene--------------------------

721  ATGATTTAGACGTGACGGCCCCCGAAGATCCCAACGAGGAGGCGGTTTCGCAGATTTTTC
     -----------------------E1a gene--------------------------

781  CCGACTCTGTAATGTTGGCGGTGCAGGAAGGGATTGACTTACTCACTTTTCCGCCGGCGC
     -----------------------E1a gene--------------------------

841  CCGGTTCTCCGGAGCCGCCTCACCTTTCCCGGCAGCCCGAGCAGCCGGAGCAGAGAGCCT
     -----------------------E1a gene--------------------------

901  TGGGTCCGGTTTCTATGCCAAACCTTGTACCGGAGGTGATCGATCTTACCTGCCACGAGG
     -----------------------E1a gene--------------------------
```

FIGURE 3B

```
961   CTGGCTTTCCACCCAGTGACGACGAGGATGAAGAGGGTGAGGAGTTTGTGTTAGATTATG
      ----------------------E1a gene--------------------------

1021  TGGAGCACCCCGGGCACGGTTGCAGGTCTTGTCATTATCACCGGAGGAATACGGGGGACC
      ----------------------E1a gene--------------------------

1081  CAGATATTATGTGTTCGCTTTGCTATATGAGGACCTGTGGCATGTTTGTCTACAGTAAGT
      ----------------------E1a gene--------------------------

1141  GAAAATTATGGGCAGTGGGTGATAGAGTGGTGGGTTTGGTGTGGTAATTTTTTTTTTAAT
      ----------------------E1a gene--------------------------

1201  TTTTACAGTTTTGTGGTTTAAAGAATTTTGTATTGTGATTTTTTTAAAAGGTCCTGTGTC
      ----------------------E1a gene--------------------------

1261  TGAACCTGAGCCTGAGCCCGAGCCAGAACCGGAGCCTGCAAGACCTACCCGCCGTCCTAA
      ----------------------E1a gene--------------------------

1321  AATGGCGCCTGCTATCCTGAGACGCCCGACATCACCTGTGTCTAGAGAATGCAATAGTAG
      ----------------------E1a gene--------------------------

1381  TACGGATAGCTGTGACTCCGGTCCTTCTAACACACCTCCTGAGATACACCCGGTGGTCCC
      ----------------------E1a gene--------------------------

1441  GCTGTGCCCCATTAAACCAGTTGCCGTGAGAGTTGGTGGGCGTCGCCAGGCTGTGGAATG
      ----------------------E1a gene--------------------------

1501  TATCGAGGACTTGCTTAACGAGCCTGGGCAACCTTTGGACTTGAGCTGTAAACGCCCCAG
      ----------------------E1a gene--------------------------

1561  GCCATAAGGTGTAAACCTGTGATTGCGTGTGTGGTTAACGCCTTTGTTTGCTGAATGAGT
      ----------------------E1a gene--------------------------

1621  TGATGTAAGTTTAATAAAGGGTGAGATAATGTTTAACTTGCATGGCGTGTTAAATGGGGC
      ----------------------+--------------------------

1681  GGGGCTTAAAGGGTATATAATGCGCCGTGGGCTAATCTTGGTTACATCTGACCTCATGGA
      ----------------------E1b gene--------------------------

1741  GGCTTGGGAGTGTTTGGAAGATTTTTCTGCTGTGCGTAACTTGCTGGAACAGAGCTCTAA
      ----------------------E1b gene--------------------------

1801  CA
      --
```

FIGURE 3C

```
33881 AACCTACGCCCAGAAACGAAAGCCAAAAAACCCACAACTTCCTCAAATCGTCACTTCCGT


33941 TTTCCCACGTTACGTCACTTCCCATTTTAATTAAGAATTCTACAATTCCCAACACATACA


34001 AGTTACTCCGCCCTAAAACCCTGGGCGAGTCTCCACGTAAACGGTCAAAGTCCCCGCGGC
                           +-packaging signal----------------------

34061 CCTAGACAAATATTACGCGCTATGAGTAACACAAAATTATTCAGATTTCACTTCCTCTTA
      --------------------packaging signal----------------------

34121 TTCAGTTTTCCCGCGAAAATGGCCAAATCTTACTCGGTTACGCCCAAATTTACTACAACA
      --------------------packaging signal----------------------

34181 TCCGCCTAAAACCGCGCGAAAATTGTCACTTCCTGTGTACACCGGCGCACACCAAAAACG
      ------------------------------------+

34241 TCACTTTTGCCACATCCGTCGCTTACATGTGTTCCGCCACACTTGCAACATCACACTTCC


34301 GCCACACTACTACGTCACCCGCCCCGTTCCCACGCCCCGCGCCACGTCACAAACTCCACC
              +----------------ITR----------------------------

34361 CCCTCATTATCATATTGGCTTCAATCCAAAATAAGGTATATTATTGATGATG
      ----------------------------ITR--------------------+
```

FIGURE 4

```
  1 CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGGGGGTGGAGT
    +------------------------ITR---------------------------------

 61 TTGTGACGTGGCGCGGGGCGTGGGAACGGGGCGGGTGACGTAGGGCGCGCCGCTAGCGAT
    --------------------------ITR--------------++------MCS--------

121 ATCGGATCCCGGTCGACTGAAAATGAGACATATTATCTGCCACGGAGGTGTTATTACCGA
    --------------+-----------E1a--------------------------------

181 AGAAATGGCCGCCAGTCTTTTGGACCAGCTGATCGAAGAGGTACTGGCTGATAATCTTCC
    --------------------------E1a--------------------------------

241 ACCTCCTAGCCATTTTGAACCACCTACCCTTCACGAACTGTATGATTTAGACGTGACGGC
    --------------------------E1a--------------------------------

301 CCCCGAAGATCCCAACGAGGAGGCGGTTTCGCAGATTTTTCCCGACTCTGTAATGTTGGC
    --------------------------E1a--------------------------------

361 GGTGCAGGAAGGGATTGACTTACTCACTTTTCCGCCGGCGCCCGGTTCTCCGGAGCCGCC
    --------------------------E1a--------------------------------

421 TCACCTTTCCCGGCAGCCCGAGCAGCCGGAGCAGAGAGCCTTGGGTCCGGTTTCTATGCC
    --------------------------E1a--------------------------------

481 AAACCTTGTACCGGAGGTGATCGATCTTACCTGCCACGAGGCTGGCTTTCCACCCAGTGA
    --------------------------E1a--------------------------------

541 CGACGAGGATGAAGAGGGTGAGGAGTTTGTGTTAGATTATGTGGAGCACCCCGGGCACGG
    --------------------------E1a--------------------------------

601 TTGCAGGTCTTGTCATTATCACCGGAGGAATACGGGGGACCCAGATATTATGTGTTCGCT
    --------------------------E1a--------------------------------
```

FIGURE 5

```
  1 CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGGGGGTGGAGT
    +------------------------ITR-------------------------------

 61 TTGTGACGTGGCGCGGGGCGTGGGAACGGGGCGGGTGACGTAGGGCGCGATCAAGCTTAT
    --------------------------ITR--------------+         +----

121 CGATACCGTCGAAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATC
    -------------------------polyA----------------------------

181 ACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTC
    -------------------------polyA----------------------------

241 ATCAATGTATCTTATCATGTCTGGATCCGCGCCGCTAGCGATATCGGATCCCGGTCGACT
    --------------------+                                  +--

301 GAAAATGAGACATATTATCTGCCACGGAGGTGTTATTACCGAAGAAATGGCCGCCAGTCT
    -------------------------E1a------------------------------

361 TTTGGACCAGCTGATCGAAGAGGTACTGGCTGATAATCTTCCACCTCCTAGCCATTTTGA
    -------------------------E1a------------------------------

421 ACCACCTACCCTTCACGAACTGTATGATTTAGACGTGACGGCCCCCGAAGATCCCAACGA
    -------------------------E1a------------------------------

481 GGAGGCGGTTTCGCAGATTTTTCCCGACTCTGTAATGTTGGCGGTGCAGGAAGGGATTGA
    -------------------------E1a------------------------------

541 CTTACTCACTTTTCCGCCGGCGCCCGGTTCTCCGGAGCCGCCTCACCTTTCCCGGCAGCC
    -------------------------E1a------------------------------

601 CGAGCAGCCGGAGCAGAGAGCCTTGGGTCCGGTTTCTATGCCAAACCTTGTACCGGAGGT
    -------------------------E1a------------------------------
```
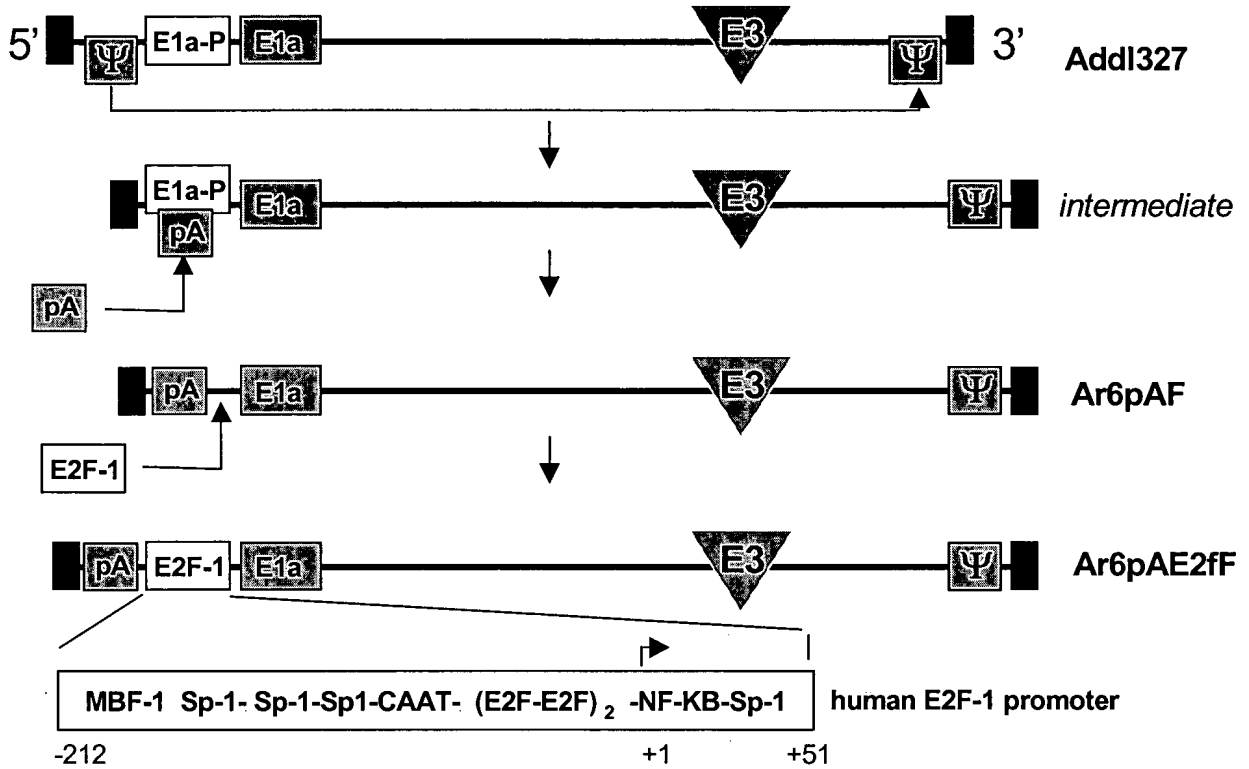
FIGURE 6

Fig. 7 Body weight change

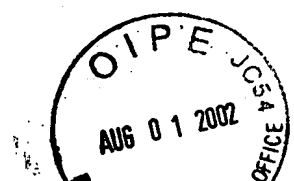# Fig. 8 Minimizing nonspecific transactivation of E1a gene

Backbones generated:
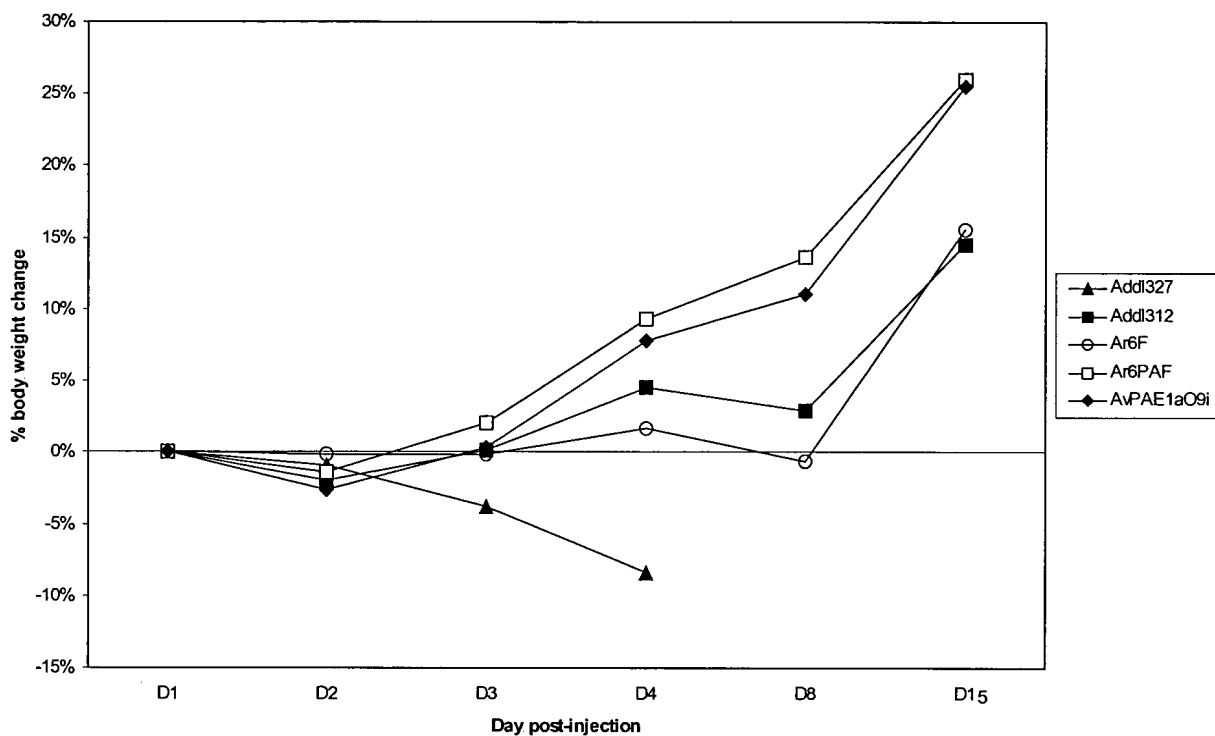
Figure 9. Mean H460 tumor volume

FIGURE 10

HBSS
Ar6pAE2fF 5e8 P
Ar6pAE2fF 5e9 P
Ar6pAE2fF 5e10 P
Addl327 5e10 P

Survival Animals(%)

Study Day

FIGURE 11

Tumor Volume (mm³)

Study Day

HBSS
Ar6pAE2fF 5E8
Ar6pAE2fF 5E9
Ar6pAE2fF 5E10
Addl327 5E10

FIGURE 12

FIGURE 13

FIGURE 14

Circular plasmid map of pDR2mGmF, 11526 bps, NPL001419, showing the following restriction sites and features:

- 11521 Sbfl
- 11063 Munl
- 10194 Stul
- 9747 Nhel
- 9458 Sphl
- 7818 BsiWl
- 6955 Srfl
- 6510 Spel
- HRF
- BstEll 1945
- Blnl 2175
- Pacl 2496
- BsrGl 2745
- Swal 2942
- Ahdl 4041
- Fspl 4266
- Pvul 4413
- Scal 4524
- AatlI 4964
- BstBl 5047
- 5Fiber
- 10000
- 2000
- 8000
- 'HRF
- loxP
- mGm
- RITRpk
- linker
- HRF

FIGURE 15

FIGURE 16

pG1NaSvBg

8948 bps

FIGURE 17

```
7878  TTCCGGACAG  ACCTCAATAA  CTCTGTTTAC  CAGAACAGGA  GGTGAGCTTA
7928  GAAAACCCTT  AGGGTATTAG  GCCAAAGGCG  CAGCTACTGT  GGGGTTTATG
7978  AACAATTCAA  GCAACTCTAC  GGGCTATTCT  AATTCAGGTT  TCTCTAGCCG
8028  GGCTGCAGGA  ATTCGATGGC  CGCTACCTAC  AATGGCCCAC  GAGAGAAAGG
                                          M   A   H      E   R    K
8078  CTAAGGTCCT  GAGGAGGATG  TGGCTGCAGA  ATTTACTTTT  CCTGGGCATT
      A   K   V    L   R   R   M    W   L   Q    N   L   L     F   L   G   I
8128  GTGGTCTACA  GCCTCTCAGC  ACCCACCCGC  TCACCCATCA  CTGTCACCCG
       V   V   Y    S   L   S    A   P   T   R    S   P   I     T   V   T
8178  GCCTTGGAAG  CATGTAGAGG  CCATCAAAGA  AGCCCTGAAC  CTCCTGGATG
      R   P   W   K    H   V   E    A   I   K    E   A   L   N     L   L   D
8228  ACATGCCTGT  CACATTGAAT  GAAGAGGTAG  AAGTCGTCTC  TAACGAGTTC
       D   M   P    V   T   L   N    E   E   V    E   V   V     S   N   E   F
8278  TCCTTCAAGA  AGCTAACATG  TGTGCAGACC  CGCCTGAAGA  TATTCGAGCA
       S   F   K    K   L   T    C   V   Q   T    R   L   K     I   F   E
8328  GGGTCTACGG  GGCAATTTCA  CCAAACTCAA  GGGCGCCTTG  AACATGACAG
       Q   G   L   R    G   N   F    T   K   L    K   G   A   L     N   M   T
8378  CCAGCTACTA  CCAGACATAC  TGCCCCCCAA  CTCCGGAAAC  GGACTGTGAA
       A   S   Y    Y   Q   T   Y    C   P   P    T   P   E    T   D   C   E
8428  ACACAAGTTA  CCACCTATGC  GGATTTCATA  GACAGCCTTA  AAACCTTTCT
       T   Q   V    T   T   Y    A   D   F   I    D   S   L    K   T   F
8478  GACTGATATC  CCCTTTGAAT  GCAAAAAACC  AGTCCAAAAA  TGAGGAAGCC
      L   T   D   I    P   F   E    C   K   K    P   V   Q   K    -
8528  CAGGCCAGCT  CTGAATCCAG  CTTCTCAGAC  TGCTGCTTTT  GTGCCTGCGT
8578  AATGAGCCAG  GAACTCGGAA  TTTCTGCCTT  AAAGGGACCA  AGAGATGTGG
8628  CACAGGTAGT  CGAATCAAGC  TTATCGATAC  CGTCGACCTC  GACTAGATAA
8678  CTTCGTATAA  TGTATGCTAT  ACGAAGTTAT  GCTAGAAATG  GACGGAATTA
8728  TTACAGAGCA  GCGCCTGCTA  GAAAGACGCA  GGGCAGCGGC  CGAGCAACAG
8778  CGCATGAATC  AAGAGCTCCA  AGACATGGTT  AACTTGCACC  AGTGCAAAA  8826
```

FIGURE 18

FIGURE 19

RH-7777, rat
hepatoma

(LD50)

- ■ Addl327 (403)
- ◄ Ar6pAE2fF (1709)
- ► Ar6pAE2fmGm (823)
- ▽ Addl312 (2595)

% Control

Particles/cell

RM-1, mouse
prostate carcinoma

(LD50)

- ■ Addl327 (572)
- ◄ Ar6pAE2fF (~2,000)
- ► Ar6pAE2fmGmF (2002)
- ▽ Addl312 (>100,000)

% Control

Particles/cell

ep 3B, human
cellular carcinoma

(LD50)

- ■ Addl327 (<0.1)
- ◄ Ar6pAE2fF (<0.1)
- ► Ar6pAE2fmGmF (<0.1)
- ▽ Addl312 (>2500)

articles/cell

H460, human
e cell carcinoma

(LD50)

- ■ Addl327 (63)
- ◄ Ar6pAE2fF (110)
- ► Ar6pAE2fmGmF (188)
- ▽ Addl312 (3594)

articles/cell

FIGURE 20

```
28536   TATTAGGCCA AAGGCGCAGC TACTGTGGGG TTTATGAACA ATTCAAGCAA
28586   CTCTACGGGC TATTCTAATT CAGGTTTCTC TAGGATCTTT CCGCAGCAGC

28636   CGCCACCATG TGGCTGCAGA GCCTGCTGCT CTTGGGCACT GTGGCCTGCA
                 M   W   L   Q   S   L   L    L   L   G   T    V   A   C

28686   GCATCTCTGC ACCCGCCCGC TCGCCCAGCC CCAGCACGCA GCCCTGGGAG
           S   I   S    A   P   A   R    S   P   S    P   S   T    Q   P   W   E

28736   CATGTGAATG CCATCCAGGA GGCCCGGCGT CTCCTGAACC TGAGTAGAGA
            H   V   N    A   I   Q    E   A   R   R    L   L   N    L   S   R

28786   CACTGCTGCT GAGATGAATG AAACAGTAGA AGTCATCTCA GAAATGTTTG
            D   T   A   A    E   M   N    E   T   V    E   V   I   S    E   M   F

28836   ACCTCCAGGA GCCGACCTGC CTACAGACCC GCCTGGAGCT GTACAAGCAG
            D   L   Q    E   P   T   C    L   Q   T    R   L   E    L   Y   K   Q

28886   GGCCTGCGGG GCAGCCTCAC CAAGCTCAAG GGCCCCTTGA CCATGATGGC
             G   L   R    G   S   L    T   K   L   K    G   P   L    T   M   M

28936   CAGCCACTAC AAGCAGCACT GCCCTCCAAC CCCGGAAACT TCCTGTGCAA
            A   S   H   Y    K   Q   H    C   P   P    T   P   E   T    S   C   A

28986   CCCAGACTAT CACCTTTGAA AGTTTCAAAG AGAACCTGAA GGACTTTCTG
            T   Q   T    I   T   F   E    S   F   K    E   N   L    K   D   F   L

29036   CTTGTCATCC CCTTTGACTG CTGGGAGCCA GTCCAGGAGT GAGTCGACAA
             L   V   I    P   F   D    C   W   E   P    V   Q   E    -

29086   GCTCTAGATA ACTTCGTATA ATGTATGCTA TACGAAGTTA TGCTAGAAAT
29136   GGACGGAATT ATTACAGAGC AGCGCCTGCT AGAAAGACGC AGGGCAGCGG
29186   CCGAGCAACA GCGCATGAAT CAAGAGCTCC AAGACATGGT TAACTTGCAC
29236   CAGTGCAAAA GGGGTATCTT TTGTCTGGTA AAGCAGG   29273
```

FIGURE 21

FIGURE 22

Hep3B, human hepatocellular carcinoma

(LD50)

☐ Addl312 (394)
○ Addl327 (<0.01)
△ Ar6pAE2fF (<0.01)
◇ Ar6pAE2fmGmF (<0.01)
✳ Ar6pAE2fhGmF (<0.01)

KLN205, mouse lung adenocarcinoma

(LD50)

☐ Addl312 (8587)
○ Addl327 (47)
△ Ar6pAE2fF (485)
◇ Ar6pAE2fmGmF (308)
✳ Ar6pAE2fhGmF (54)

H460, human large cell carcinoma

(LD50)

☐ Addl312 (2282)
○ Addl327 (255)
△ Ar6pAE2fF (489)
◇ Ar6pAE2fmGmF (717)
✳ Ar6pAE2fhGmF (64)

CMT-93, mouse rectal carcinoma

(LD50)

☐ Addl312 (15150)
○ Addl327 (332)
△ Ar6pAE2fF (422)
◇ Ar6pAE2fmGmF (336)
✳ Ar6pAE2fhGmF (56)

FIGURE 23

B. H460 anti-tumor efficacy using 1×10^{11} particles/injection

Tumor Volume, mm^3

PBS
Ad dl312
Ad dl327
Ar6pAE2fF
Ar6pAE2fmGmF

OV Injections, it.

Days Post-H460 Injection

A. H460 anti-tumor efficacy using 2×10^{10} particles/injection

Tumor Volume, mm^3

PBS
Ad dl312
Ad dl327
Ar6pAE2fF
Ar6pAE2fmGmF

OV Injections, it.

Days Post-H460 Injection

FIGURE 24

FIGURE 25

Primer1

Primer2

Primer3

Primer4

Primer5

Primer6

NotI

Primer6

E3

GM-CSF

E3

Primer1

BsiWI

FIGURE 26A

cgccacccaagATGAttaggtac (Seq ID NO:9)

6.7kDa---------R H P R **STOP**

M------gp19kDa

(Seq ID NO:13)

FIGURE 26B

1. Δgp19a: atgg

    6.7kDa—plus 9aa to **STOP** M——mouse GMCSF

                  atgt

    6.7kDa—plus 33aa to **STOP** M——human GMCSF

2. Δgp19b: cgccacccaag ATAACCatg...... (SEQ ID NO:98)

    6.7kDa——R H P R **STOP**   M——GMC:

3. Δgp19c: cgccacccaagatgA......(Seq ID NO:10)

    6.7kDa——R H P R **STOP**

                      M——GMCSF

4. Δgp19d: cgccacccaagATGACCatg......(Seq ID NO:11)

    6.7kDa——R H P R **STOP**

                  M T M——GMCSF

5. Δgp19b/IRES: cgccacccaagatgaCAATTC...IRES...atg...... (Seq ID NO:12)

    6.7kDa——R H P R **STOP**   M——GMCSF

FIGURE 27A

FIGURE 27B

pDR2(E3+, hGm, Dg19b)F

12713 bps

EcoRV66
HRF
5Fiber
HRF
loxP
E3
hGm
E3
7818.BsrGI
8401.BsrGI
8895.NotI
9177.XhoI
10645.SphI
10934.NheI
linker
Mlu.6062
6955.SgfI
6721.EcoRV
6510.SpeI
Pac2496
BsiG.8745
Swa.8942
Cla2950

Fsp I/Spe I

Srf I/Pac I

Co-transform → **E.coli BJ5183 cells**

pAr6pAE2f(E3+,hGm, Dg19b)F

38950 bps

Bst1107I, 5808
Swa I, 9
BstBI
38377, BstBI
35825, PacI
HRF
5Fiber
loxP
hGm
27571.SrfI
21482. SgfI
L4
hexon
L2 L1
PmeI, 13299
p(A)
ITR
E2f
E1a E1b HRF

pAR6pAE2fF

37114 bps

Bst1107I, 5808
Swa I 9
36541 BstBI
33989, PacI
HRF
5Fiber
loxP
"HRF
27571, SrfI
21482, SgfI
L4
hexon
L2 L1
PmeI, 13299
p(A)
ITR
RITRpk
E2f E1a E1b HRF
dl327'

FIGURE 28

Hep3B, human
hepatocellular carcinoma

(LD50)

□ Addl312 (361)
○ Addl327 (0.0055)
△ Ar6pAE2fF (0.0022)
◇ Ar6pAE2fmGmF (0.0093)
✳ Ar6pAE2f(E3+,mGm,Dg19a)F (0.0680)
✕ Ar6pAE2f(E3+,mGm,Dg19b)F (0.0053)
✳ Ar6pAE2f(E3+,mGm,Dg19c)F (0.0362)
- Ar6pAE2f(E3+,mGm,Dg19d)F (0.0558)
✦ Ar6pAE2f(E3+,lmGm,Dg19b)F (0.0327)

Hep3B, human
hepatocellular carcinoma

(LD50)

□ Addl312 (>625)
○ Addl327 (<0.025)
△ Ar6pAE2fF (<0.025)
◇ Ar6pAE2fhGm (<0.025)
✳ Ar6pAE2f(E3+,hGm,Dg19a)F (<0.025)
✕ Ar6pAE2f(E3+,hGm,Dg19b)F (<0.025)
✳ Ar6pAE2f(E3+,hGm,Dg19c)F (0.055)

H460, human
large cell carcinoma

(LD50)

□ Addl312 (>10,000)
○ Addl327 (324)
△ Ar6pAE2fF (253)
◇ Ar6pAE2fhGmF (621)
✳ Ar6pAE2f(E3+,mGm,Dg19a)F (1301)
✕ Ar6pAE2f(E3+,mGm,Dg19b)F (513)
✳ Ar6pAE2f(E3+,mGm,Dg19c)F (1127)
- Ar6pAE2f(E3+,mGm,Dg19d)F (708)
✦ Ar6pAE2f(E3+,lmGm,Dg19b)F (1184)

H460, human
large cell carcinoma

(LD50)

□ Addl312 (>10,000)
○ Addl327 (323)
△ Ar6pAE2fF (838)
◇ Ar6pAE2fhGmF (624)
✳ Ar6pAE2f(E3+,hGm,Dg19a)F (530)
✕ Ar6pAE2f(E3+,hGm,Dg19b)F (589)
⬟ Ar6pAE2f(E3+,hGm,Dg19c)F (355)

Figure 29

a. Mouse GM-CSF expression in H460 cells

Legend:
- 1000ppc, 24hr
- 1000ppc, 48hr
- 100ppc, 24hr
- 100ppc, 48hr
- 10ppc, 24hr
- 10ppc, 48hr

X-axis labels:
Ar6pAE2fF
Ar6pAE2fmGmF
Ar6pAE2f(E3+, mGm, Dg19a)F
Ar6pAE2f(E3+, mGm, Dg19b)F
Ar6pAE2f(E3+, mGm, Dg19c)F
Ar6pAE2f(E3+, mGm, Dg19d)F
Ar6pAE2f(E3+, lmGm, Dg19b)F

Y-axis: ng mGM-CSF/ Million cells

b. Human GM-CSF expression in H460 cells

Legend:
- 1000ppc, 24hr
- 1000ppc, 48hr
- 100ppc, 24hr
- 100ppc, 48hr
- 10ppc, 24hr
- 10ppc, 48hr

X-axis labels:
pAE2fF
fhGmF
19a)F
19b)F
19c)F

Y-axis: ng hGM-CSF/ Million cells

Figure 30

Figure 31

Figure 32

A. pDr5hGmF

B. pDr5mGmF

Figure 33

Figure 34

Figure 35

H460, human large cell carcinoma

(LD50)

☐ Addl312 (8631)
○ Addl327 (444)
△ Ar6pAE2fmGmF (517)
◇ Ar6pAE2f(E3+,mGm,Dg19b)F (392)
✳ Ar15pAE2fF (288)
✕ Ar15pAE2fmGmF (355)
✱ Ar15pAE2fhGmF (294)

Hep3B, human hepatocellular carcinoma

(LD50)

☐ Addl312 (1098)
○ Addl327 (0.0356)
△ Ar6pAE2fmGmF (0.1457)
◇ Ar6pAE2f(E3+,mGm,Dg19b)F (0.0005)
✳ Ar15pAE2fF (0.0003)
✕ Ar15pAE2fmGmF (0.0094)
✱ Ar15pAE2fhGmF (0.0320)

Figure 36

Figure 37

Primer1

Primer2

Primer3

Primer4

Primer5

Primer6

Primer1

'E3   'HRF

Xho I   Primer6

E3 14.5K   hGM-CSF   5Fiber'   Sph I

200   400   600   800   1000   1200   1400

E3-hGm/Xhol + SphI   (1491 bps)

Figure 38A

ggaggagATGacTGAttaggtac (Seq ID NO:14)

E3 14.5kDa--------G G D D (STOP) (Seq ID NO:16)

M------E3 14.7kDa

Figure 38B

ggaggagæcgacTGACC atg......... (Seq ID NO:15)

E3 14.5kDa-------G G D D (STOP)   M------GMCSF

Figure 39

pDR6hGmF

12774 bps

NPL002858

HRF

'5Fiber \

12000

2000

10706, Sphl

hGm

10000

E3 14.5K

9219, Xhol

8937, Notl

E3

8020, Xbal
7818, BsiWI

6955, Srfl

6510, Spel

6000

linker

PacI, 2496

SwaI, 2942
ClaI, 2950

FspI, 4266

MluI, 5062

Figure 40

Figure 41

H460,human large cell carcinoma

(LD50)

□	Addl312 (3659)
○	Addl327 (417)
△	Ar6pAE2fF (916)
◇	Ar6pAE2fhGmF (639)
✳	Ar6pAE2f(E3+,hGm,Dg19b)F (421)
✲	Ar16pAE2fhGmF (899)

Particles/cell

% Control

Figure 42

Figure 43

A. Day 1

B. Day 4

C. Day 7

Legend:
◇ PBS
□ Ad*dl*312
△ Ar6pAE2fF
○ Ar6pAE2fE3F

y-axis: % hexon positive cells (60, 50, 40, 30, 20, 10, 0)
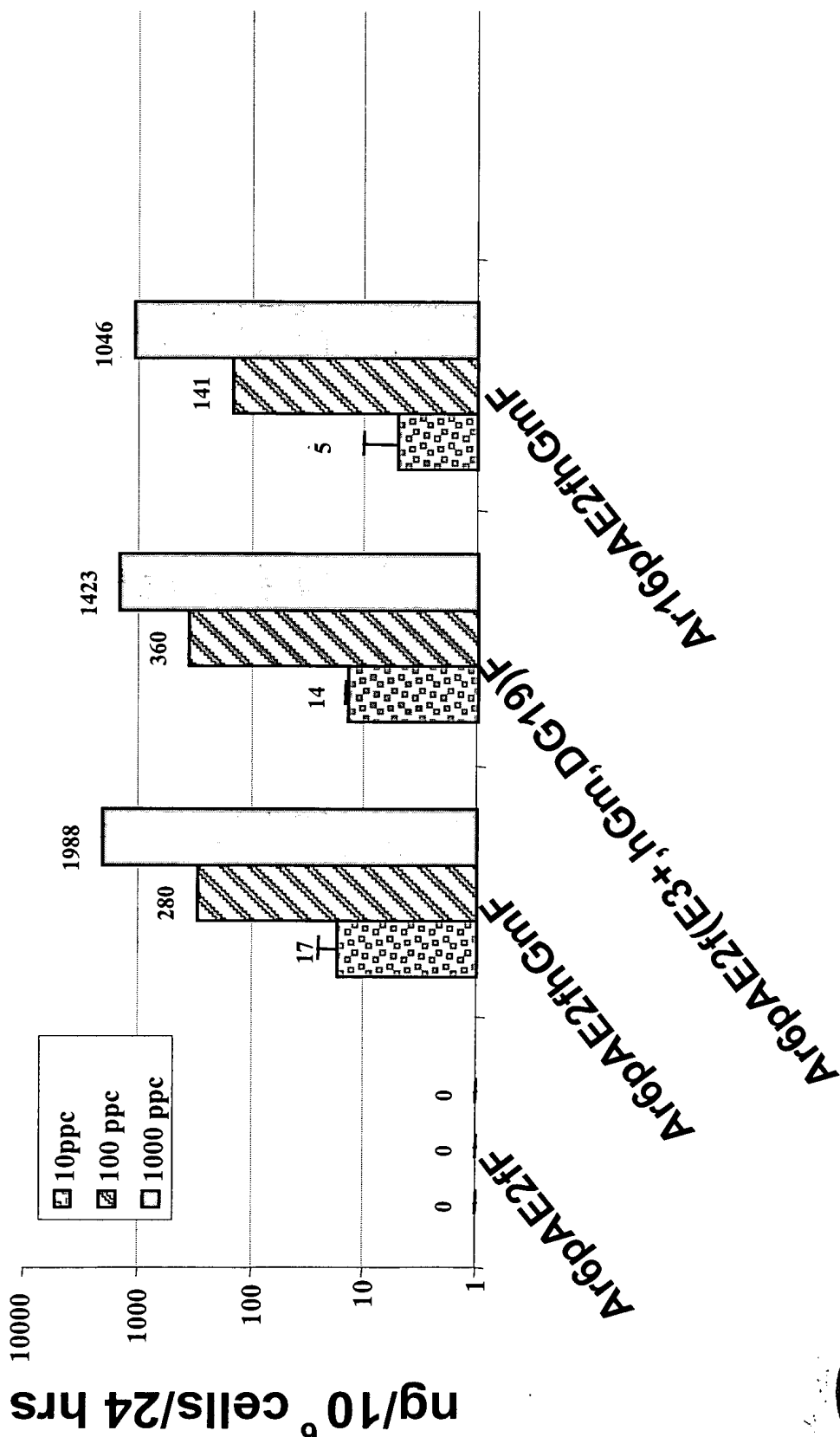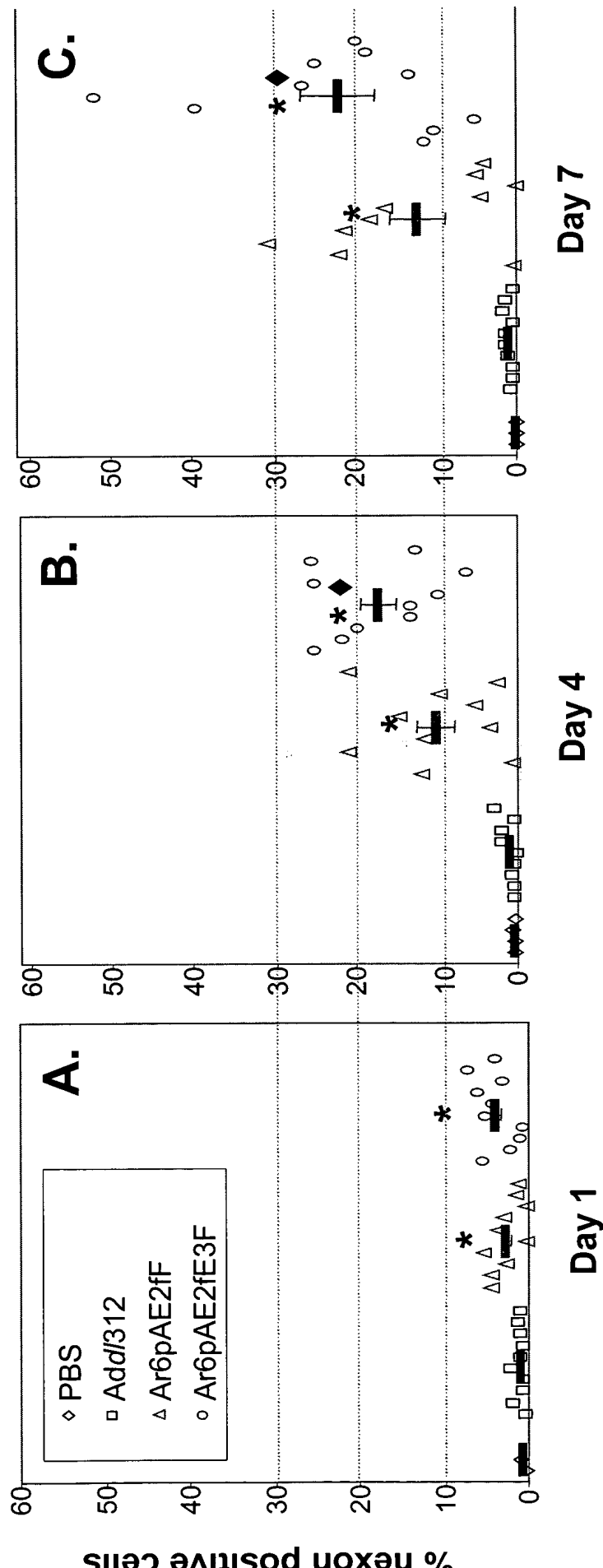
p<0.05 between Ar6pAE2fF or Ar6pAE2fE3F and Ad*dl*312, ANOVA
p<0.05 between Ar6pAE2fF and Ar6pAE2fE3F vectors, ANOVA

Figure 44

A.

| PBS |
| Add*l*312 |
| Ar6pAE2fGmF |
| Ar6pAE2f(E3+,GmDg19)F |

Day 1

B.

Day 4

C.

Day 7

% hexon positive cells
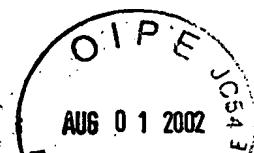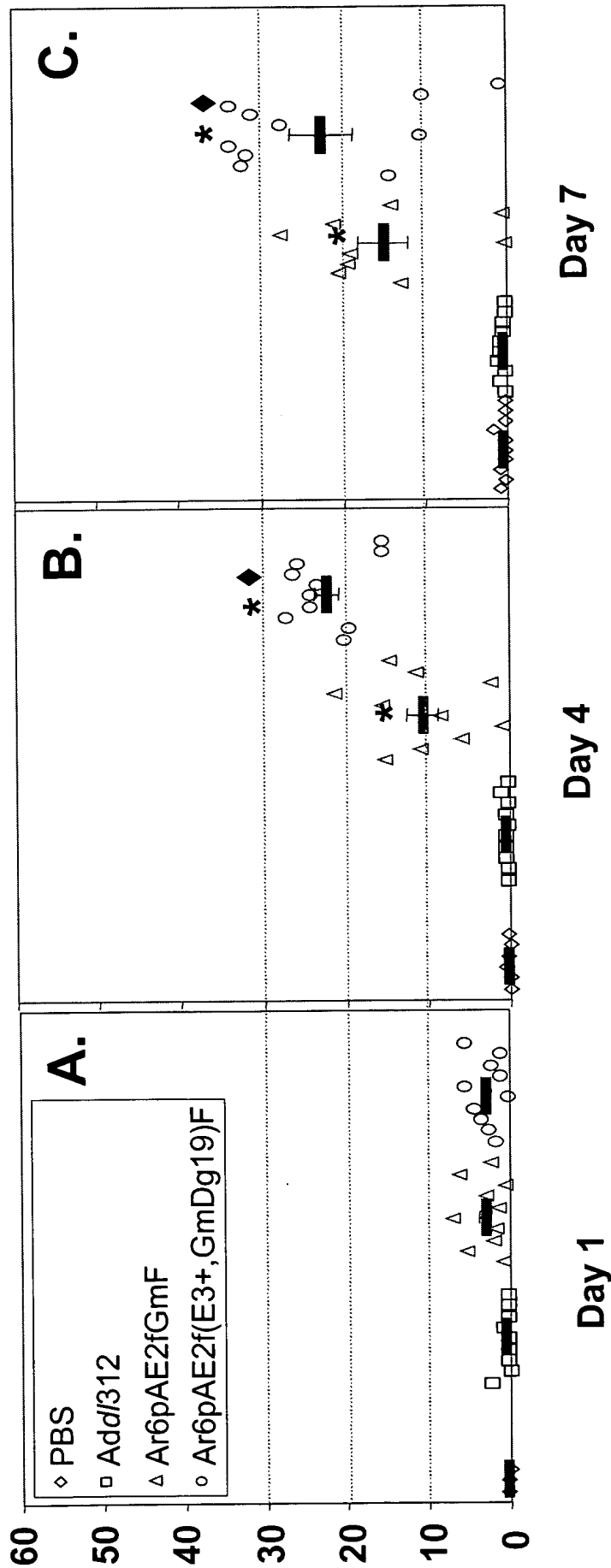
p<0.05 between Ar6pAE2fhGmF or Ar6pAE2f(E3+,hGm,Dg19)F and Add*l*312, ANOVA
p<0.05 between Ar6pAE2fhGmF and Ar6pAE2f(E3+,hGm,Dg19)F vectors, ANOVA

Figure 45

Figure 46

Figure 47

```
35351   agtgctaaaa agcgaccgaa atagcccggg ggaatacata cccgcaggcg
35401   tagagacaac attacagccc ccataggagg tataacaaaa ttaataggag
35451   agaaaaacac ataaacacct gaaaaaccct cctgcctagg caaaatagca
35501   ccctcccgct ccagaacaac atacagcgct tcacagcggc agcctaacag
35551   tcagccttac cagtaaaaaa gaaaacctat taaaaaaaca ccactcggat
35601   caattcgcgg gggtggccgg ggccagggct tcccacgtgc gcagcaggac
35651   gcagcgctgc ctgaaactcg cgccgcgagg agagggcggg gccgcggaaa
35701   ggaaggggag gggctgggag ggcccggagg gggctgggcc ggggacccgg
35751   gaggggtcgg gacggggcgg ggtccgcgcg gaggaggcgg agctggaagg
35801   tgaaggggca ggacgggtgc ccgggtcccc agtccctccg ccacgtgggg
35851   ctaggatcct taattaagaa ttctacaatt cccaacacat acaagttact
35901   ccgccctaaa accctgggcg agtctccacg taaacggtca aagtccccgc
35951   ggccctagac aaatattacg cgctatgagt aacacaaaat tattcagatt
36001   tcacttcctc ttattcagtt ttcccgcgaa aatggccaaa tcttactcgg
36051   ttacgcccaa atttactaca acatccgcct aaaaccgcgc gaaaattgtc
36101   acttcctgtg tacaccggcg cacaccaaaa acgtcacttt tgccacatcc
36151   gtcgcttaca tgtgttccgc cacacttgca acatcacact tccgccacac
36201   tactacgtca cccgccccgt tcccacgccc cgcgccacgt cacaaactcc
36251   accccctcat tatcatattg gcttcaatcc aaaataaggt atattattga
36301   tgatg
```

Figure 48

E box   3 x SP1   8 x AP2   2 x SP1   E box

MBF-1 3xSP1 CAAT (E2F-E2F)2 NF-kB SP1

ITR

3'

Packaging signal

hTrtex promoter

+E3 wild type

E4

E1a

pA

E2F-1 promoter

5'

Figure 49

A Ar17pAE2fFTrtex

Figure 50

```
35521  ATACAGCGCT TCACAGCGGC AGCCTAACAG TCAGCCTTAC CAGTAAAAAA GAAAACCTAT
            ExtP1

35581  TAAAAAAACA CCACTCGGAT CAATTCGCGG GGGTGCCCGG GGCCAGGGCT TCCCACGTGC

35641  GCAGCAGGAC GCAGCGCTGC CTGAAACTCG CGCCGCGAGG AGAGGGCGGG GCCGCGGAAA

35701  AGGAACGGGA CGGGCTGGGA TGGCCCGGAA GGGGCTGGGC CGGGGACCCG GGAAGGGTTC

35761  GGGACGGGGC GGGGTTCCGC GCGGACGAGG CGGAGCTGGA AGGTGAAGGG GCAGGACCGG

35821  TGCCCGGGTC CCCAGTCCCT CCGCCACGTG GGGCTAGGAT CCTTAATTAA GAATTCTACA

35881  ATTCCCAACA CATACAAGTT ACTCCGCCCT AAAACCCTGG GCG
```

Figure 51

Figure 52

- □ HBSS
- △ dl312
- ▽ Ar17pAE2fTrtex 1.5e12
- ◆ Ar17pAE2fTrtex 3e12
- ● Ar17pAE2fTrtex 4.5e12

Percent Survival vs Study Day

Y-axis: Percent Survival (1.00, 0.75, 0.50, 0.25, 0.00)
X-axis: Study Day (0, 10, 20, 30, 40, 50)

Figure 53

Figure 54

1   81969.080102

Figure 55

Legend:
- ◆ HBSS
- ■ Addl312 4.5E12
- ▲ Ar17pAE2fFTrtex 3E12
- ✕ Ar17pAE2fFTrtex 4.5E12

X-axis: study day (0, 10, 20, 30, 40, 50, 60, 70)
Y-axis: Percent Survival (0, 50, 100)

Figure 56

Figure 57

Figure 58

A)

B)

C)

D)

E)

Figure 59

HBSS
Ar17pAE2fFTrtex 3.0E+11
Ar17pAE2fFTrtex 6.0E+11
Ar17pAE2fFTrtex 1.0E+12
Ar17pAE2fFTrtex 3.0E+12
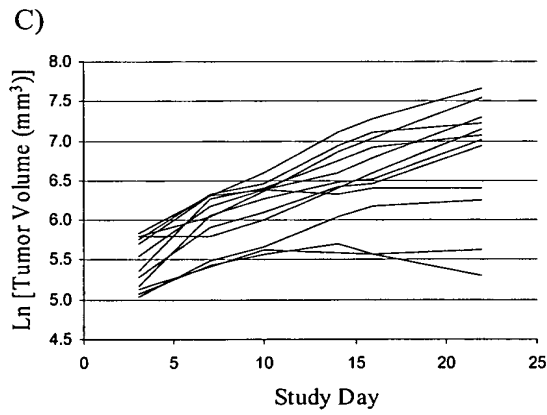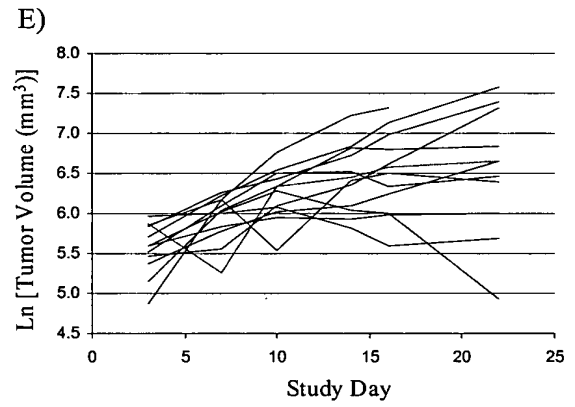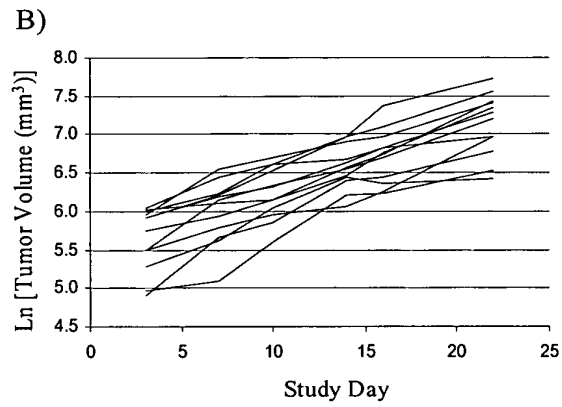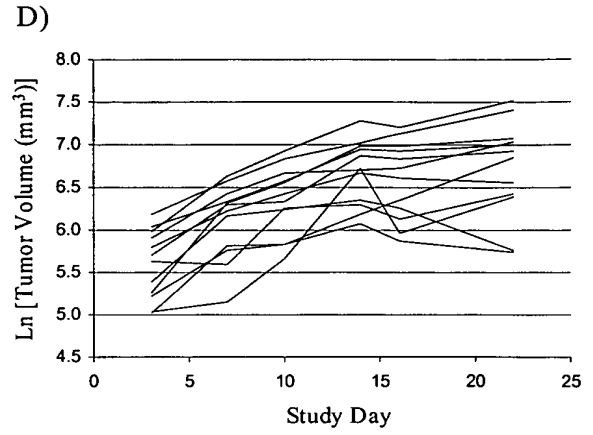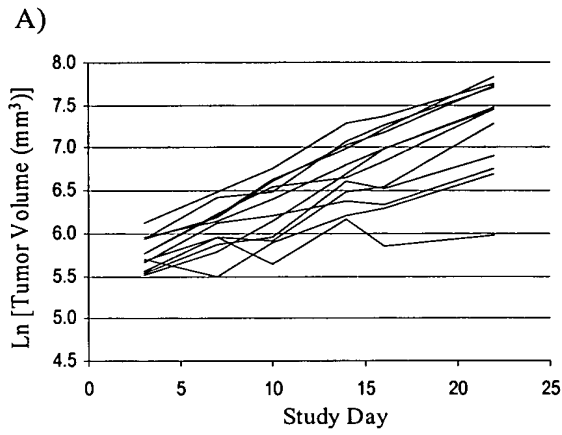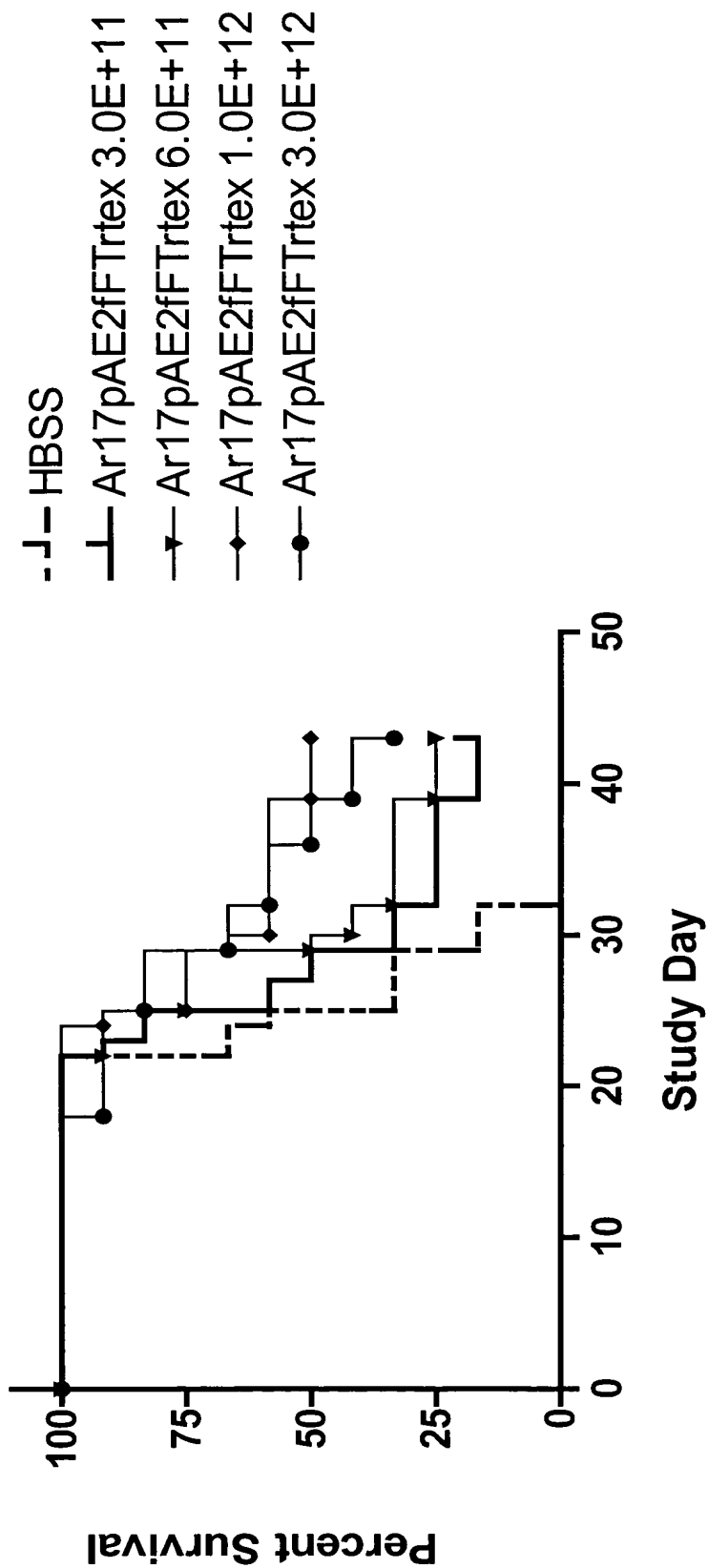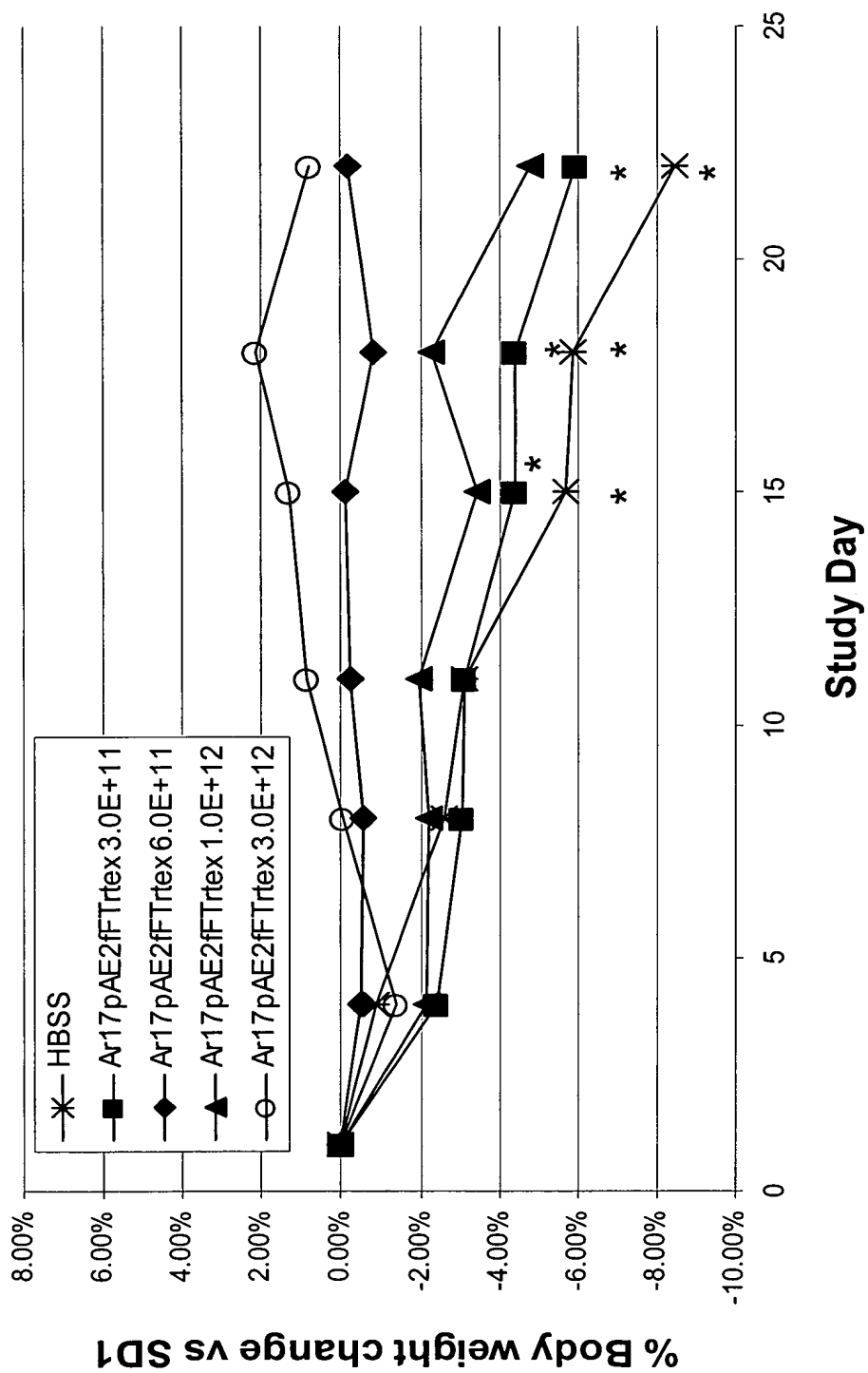
Figure 60

Figure 61

Figure 62

FIGURE 63

Hep3B

Axes: Taxol EC$_{50}$ (nM) vs Ar17pAE2fFTrtex EC$_{50}$ (ppc)

Legend: Mode I, Mode IIa, Mode IIb

PC3M2AC6 cells

Axes: Taxol EC$_{50}$ (nM) vs Ar17pAE2fFTrtex EC$_{50}$ (ppc)

Legend: Mode I, Mode IIa, Mode IIb
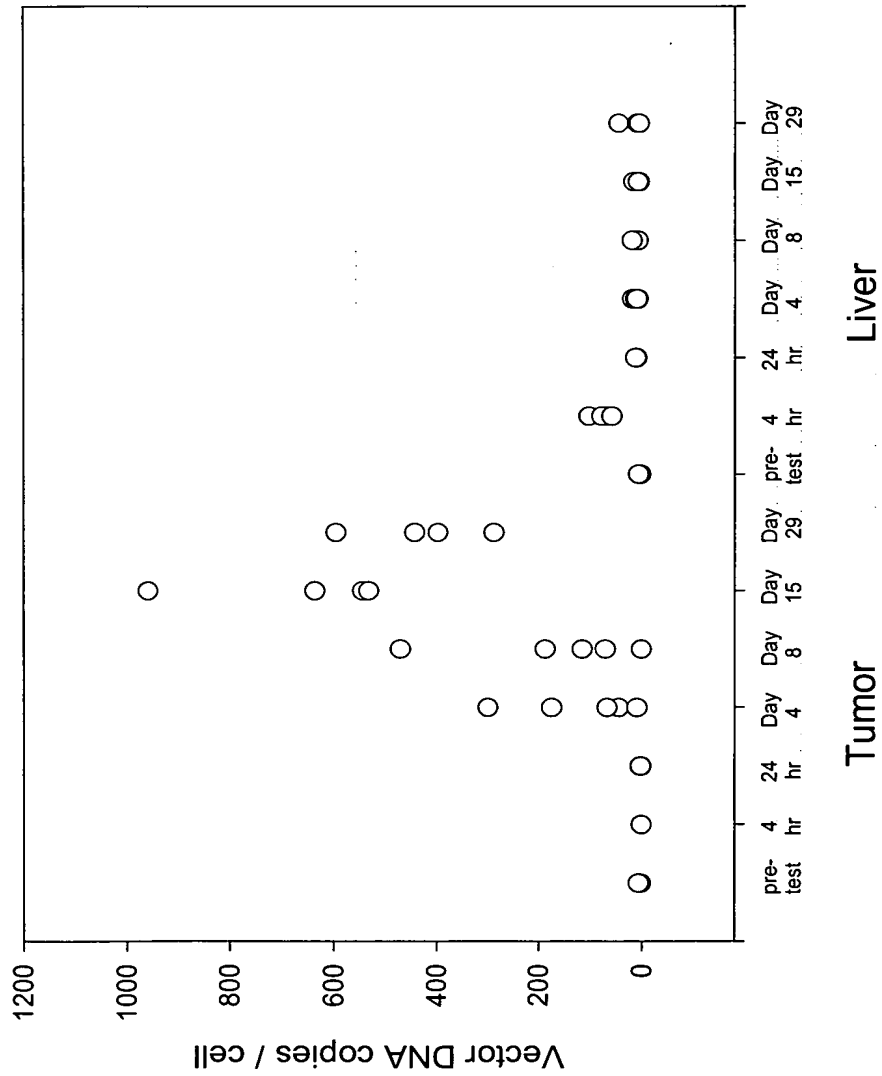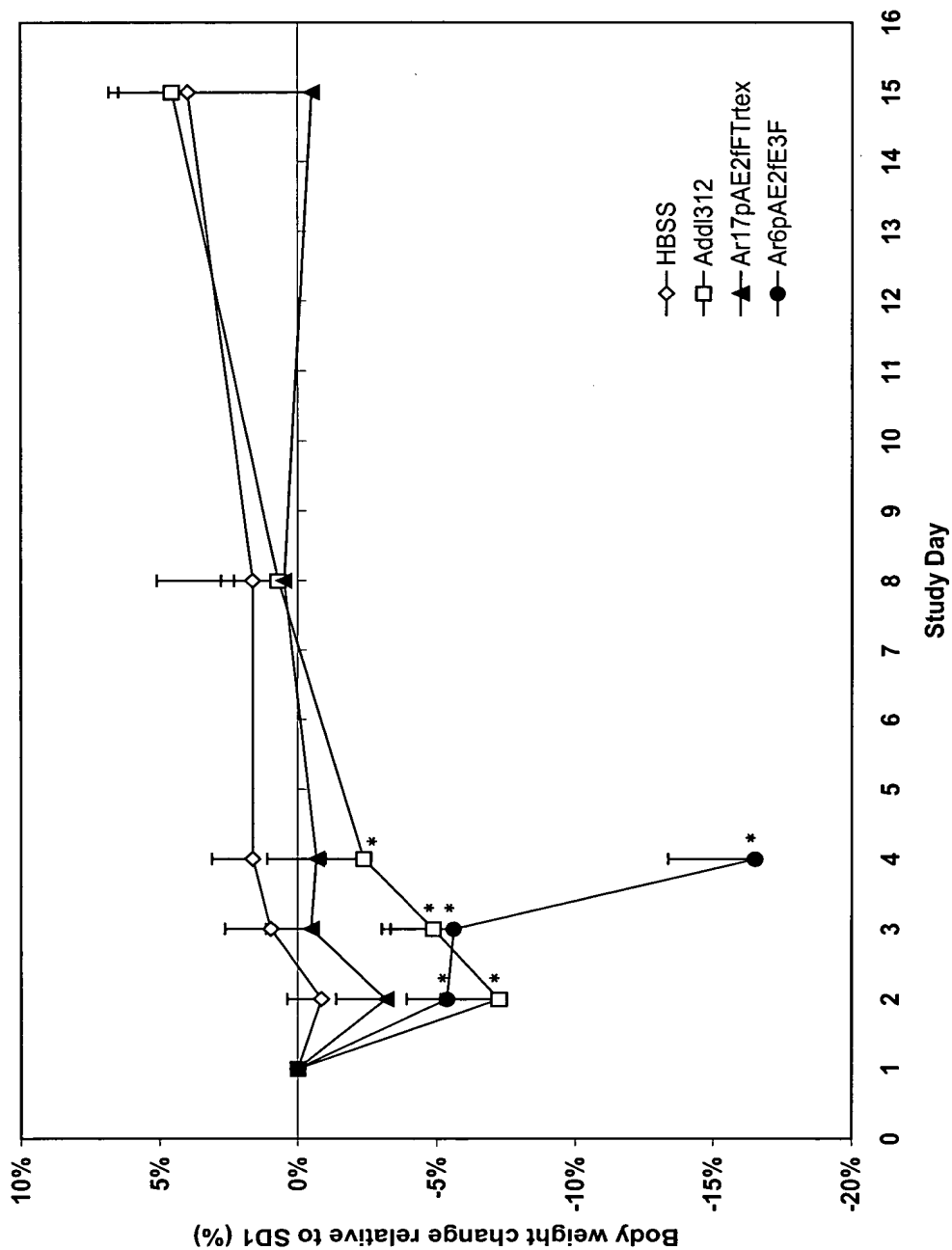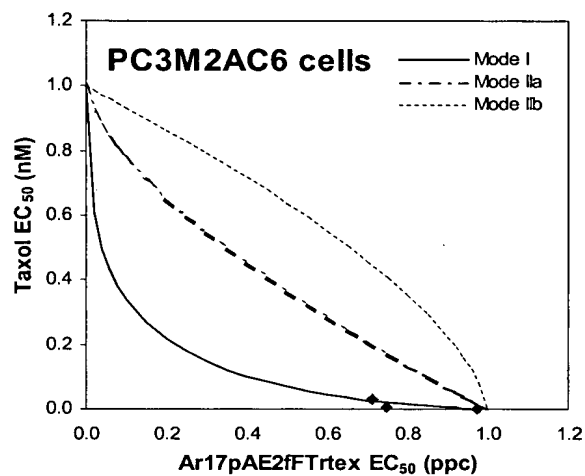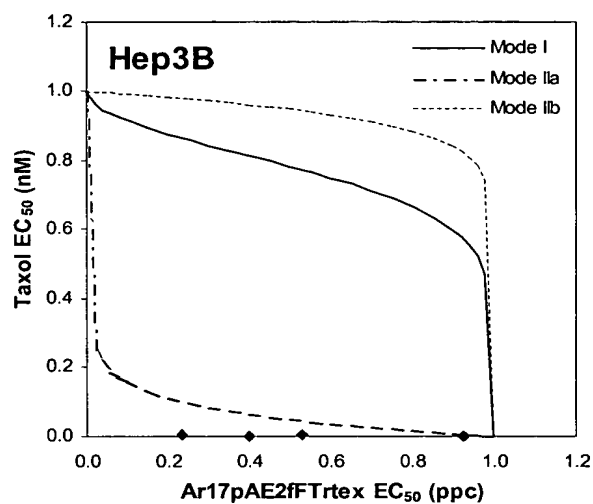
MR (ppc/nM)	Virus EC$_{50}$[b]	Chemo EC$_{50}$[b]	Effect
Virus alone	1	0	-
Chemo alone	0	1	-
8.3e-05	0.23	0.0043	synergy
3.3e-04	0.53	0.0024	synergy
1.3e-03	0.40	0.00046	synergy
5.3e-03	0.93	0.00027	synergy

MR (ppc/nM)	Virus EC$_{50}$[b]	Chemo EC$_{50}$[b]	Effect
Virus alone	1	0	-
Chemo alone	0	1	-
0.02	3.4	1.3	antagonism
0.2	0.71	0.028	synergy
2	0.75	0.003	synergy
20	0.97	0.0004	synergy

Figure 64

Hep3B — Doxorubicin EC_{50} (nM) vs Ar17pAE2fFTrtex EC_{50} (ppc); Mode I, Mode IIa, Mode IIb

PC3M2AC6 — Doxorubicin EC_{50} (nM) vs Ar17pAE2fFTrtex EC_{50} (ppc); Mode I, Mode IIa, Mode IIb
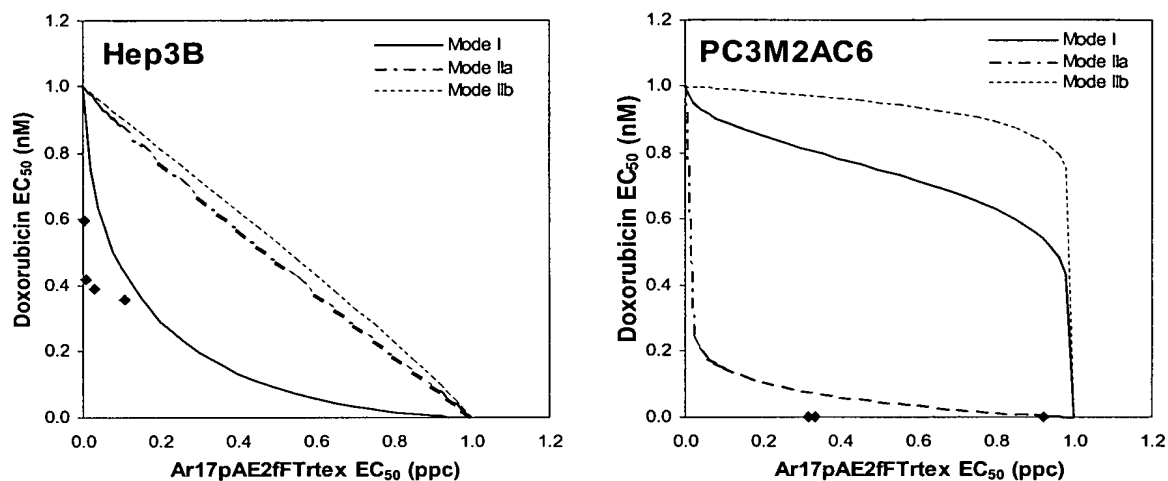
MR (ppc/nM)	Virus EC_{50}[b]	Chemo EC_{50}[b]	Effect
Virus alone	1	0	-
Chemo alone	0	1	-
1.3e-05	0.0028	0.60	synergy
5.0e-05	0.0078	0.42	synergy
2.0e-04	0.029	0.39	synergy
8.0e-04	0.11	0.36	synergy

MR (ppc/nM)	Virus EC_{50}[b]	Chemo EC_{50}[b]	Effect
Virus alone	1	0	-
Chemo alone	0	1	-
1	2.2	0.015	antagonism
10	0.92	6.1e-4	synergy
100	0.34	2.2e-5	synergy
1000	0.32	2.1e-6	synergy

Figure 65
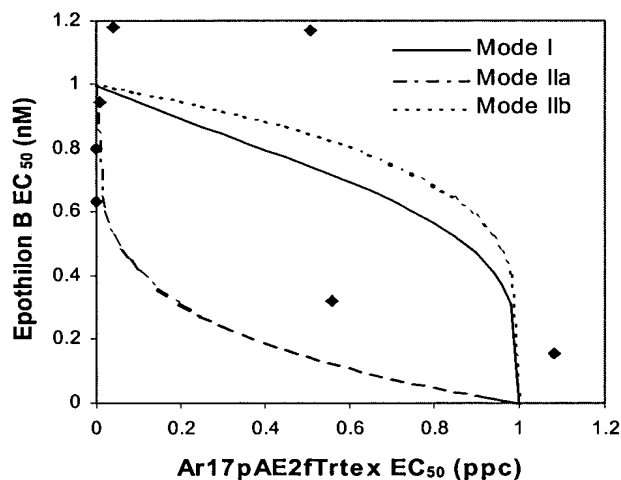
	Virus EC_{50}^b	Chemo EC_{50}^b	Effect
Virus alone	1	0	-
Chemo alone	0	1	-
3.1e-06	0.00045	0.63	synergy
1.3e-05	0.0018	0.80	synergy
5.0e-05	0.0084	0.95	synergy
2.0e-04	0.042	1.2	antagonism
8.0e-04	0.18	1.6	antagonism
3.2e-03	0.51	1.2	antagonism
1.3e-02	0.56	0.32	additivity
5.1e-02	1.1	0.06	antagonism

Figure 66

Figure 67

X-axis: **Study Day** (0, 5, 10, 15, 20, 25, 30)

Y-axis: **Mean Tumor Volume (mm3)** (0, 1000, 2000, 3000, 4000)

Legend:
- ◆ HBSS
- ■ Ar17pAE2fFTrtex 1e12 vp/Kg
- ◀ DOXIL 9mg/Kg
- ○ DOXIL+Ar17pAE2fFTrtex 1e+12 vp/kg
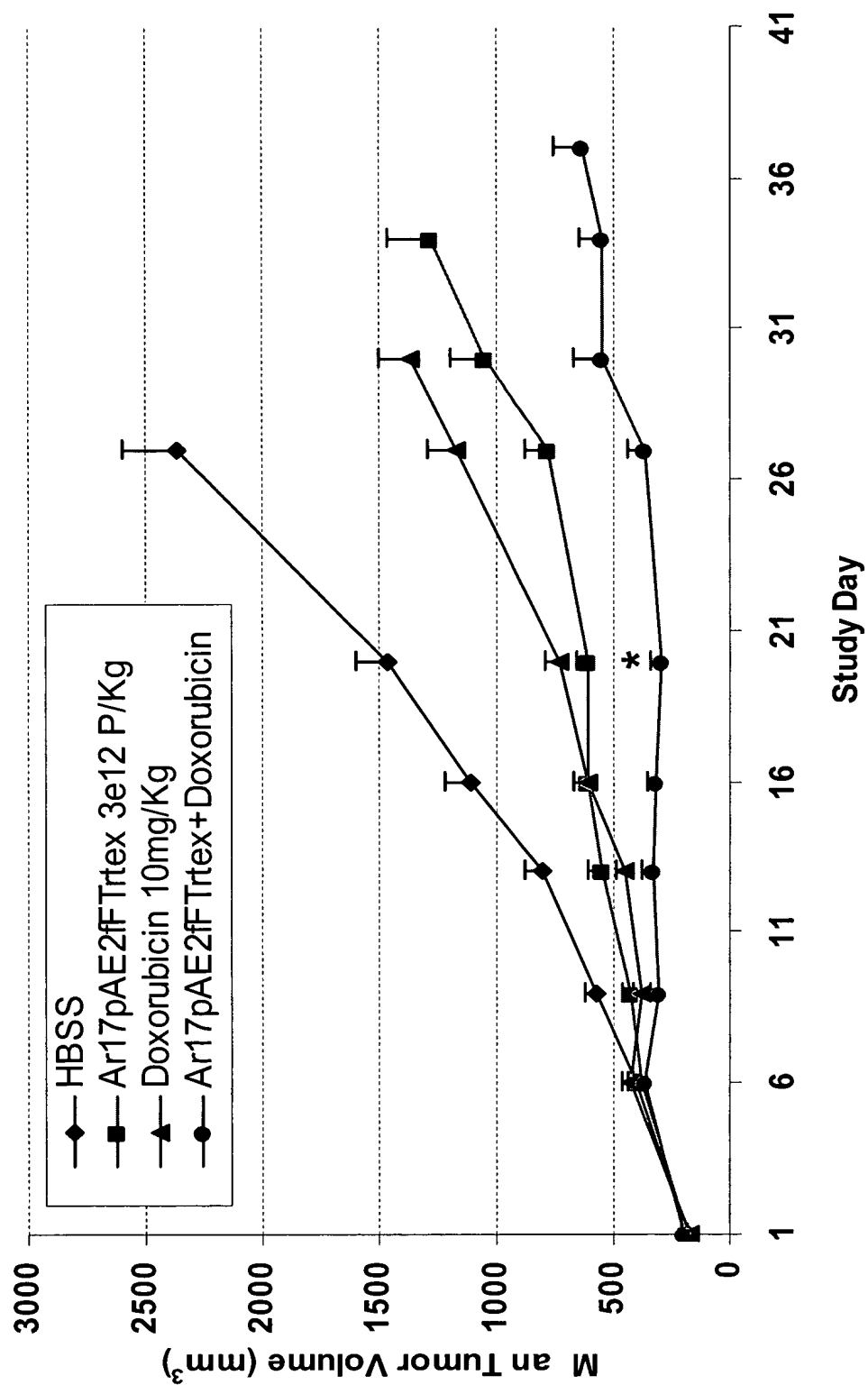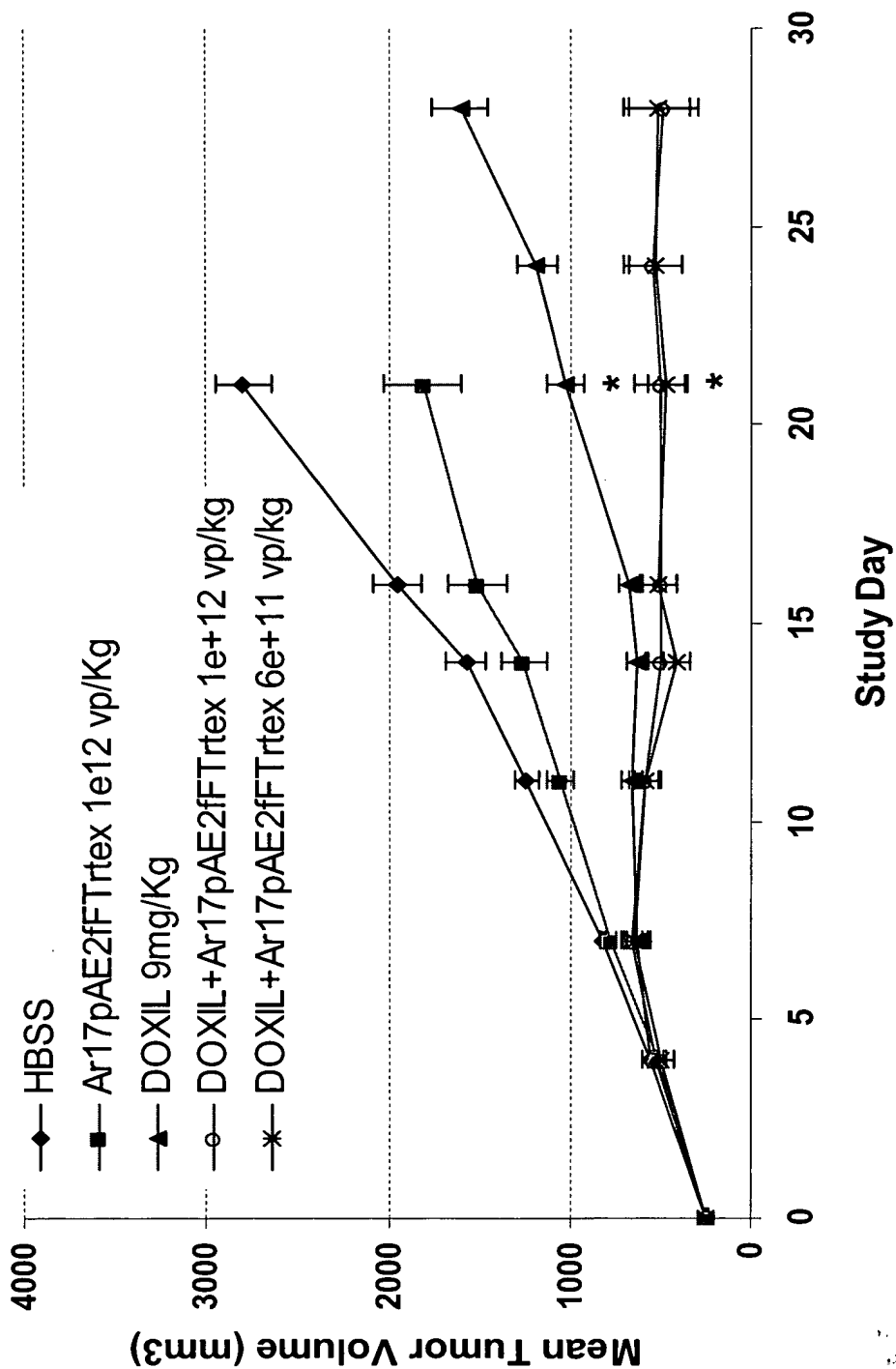- ✳ DOXIL+Ar17pAE2fFTrtex 6e+11 vp/kg
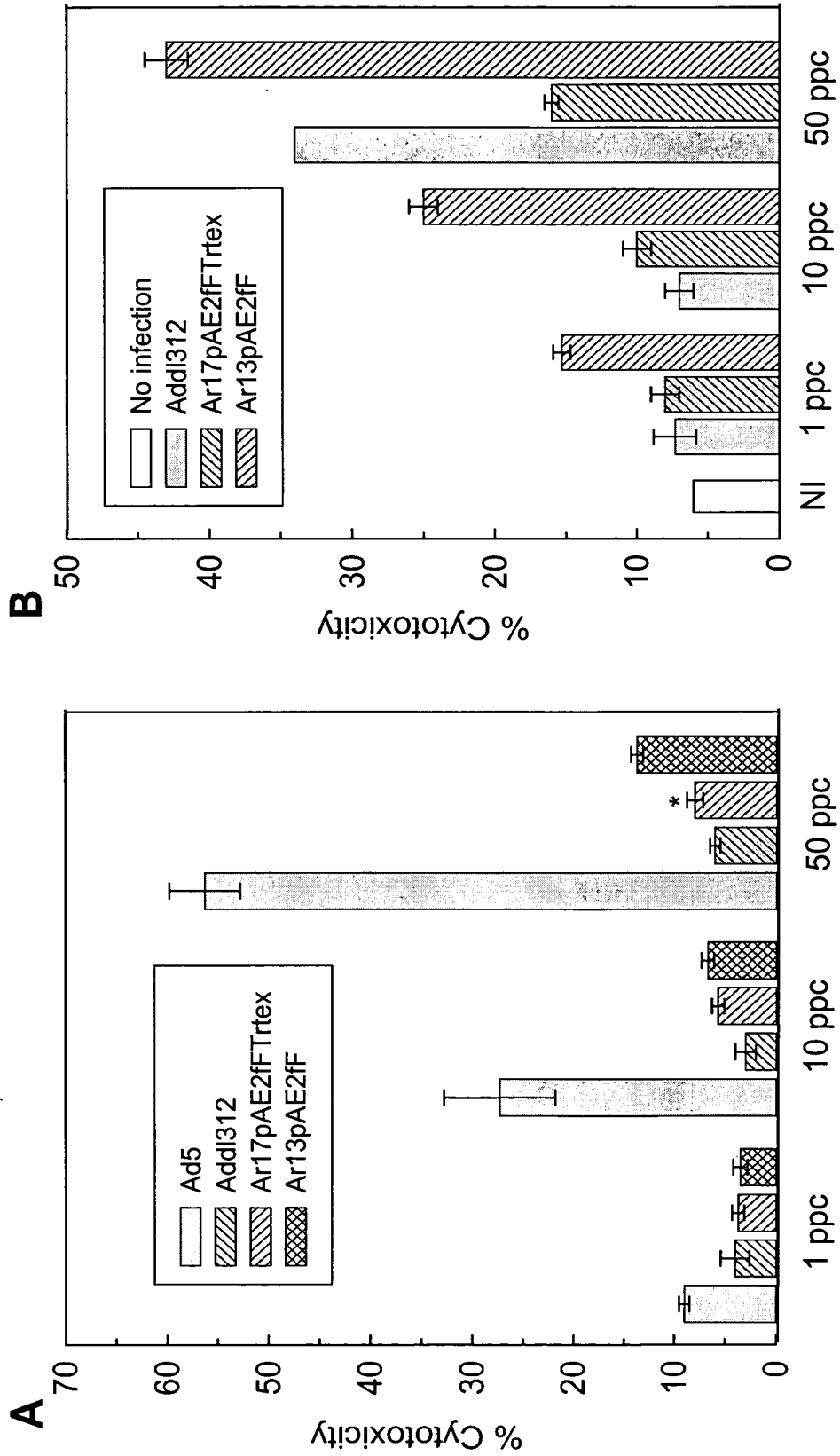
10/031969.080102

Figure 68

A

B

Figure 69

Ad35-Based Oncolytic Vectors

Figure 70